PIEBALD RATS AND MULTIPLE FACTORS

E. C. MacDOWELL

Station for Experimental Evolution, Cold Spring Harbor, L. I.

Introduction

The experiments of Castle and Phillips (:14) with piebald rats afford the largest mass of recorded data on the influence of selection in mammals. For 17 generations, the area of pigmentation on their hooded rats has been increasingly modified. In one line (the plus race) the pigmentation has been extended; in the other line (the minus race) it has been reduced. When rats from the plus or minus race are crossed with fully pigmented rats, such as the normal wild, or the Irish variety, the hooded pattern behaves as a simple Mendelian recessive, disappearing in the first generation and reappearing in one fourth of the offspring in the second generation. These results lead to the conclusion that hoodedness appears when a certain germinal unit, or factor, is in a zygote in a homozygous condition. Besides this, Castle concludes that the factor determining hoodedness fluctuates, and, in accord with its fluctuations, the amount of hooding varies. It follows that the selection of extreme grades of hoodedness results in the simultaneous selection of extreme variations of the factor. Moreover, Castle (:16, p. 722) finally concludes that the selection of these extreme grades of hoodedness influences the direction in which the factor for hoodedness varies.

These conclusions bear on one of the most generally interesting and vital questions before biologists. If, besides deciding which individuals shall mature and reproduce, selection can influence the direction in which the units of inheritance, or factors, vary, there can be no question but that

selection, as an agency in evolution, must then be restored to the important place which it held in Darwin's estimation, an agency capable of producing continuous and progressive racial changes (Castle, 15*b*, p. 97).

Castle's experiments have justly become famous. For eight years they have been continuously in progress; they have involved large amounts of arduous labor; they have been conducted with unflagging zeal and high ideals of scientific attainment. The conclusions drawn from such an important investigation should receive painstaking consideration.

The writer has been conducting selection experiments which have led him to conclusions different from those reached by Castle. Although these experiments have not involved the expenditure of so much labor and time as did Castle's work, they include three times as many generations and four times as many individuals as are reported by Castle. One investigation was on rats, the other on flies, yet there are so many similarities in the results that the writer was led to make a careful analysis of Castle's papers in an attempt to discover the basis for the conflicting conclusions. The final result of this study was to make the writer feel that the following statements in regard to the hooded rats are too positive.

All the evidence we have thus far obtained indicates that outside modifiers will not account for the changes observed in the hooded pattern, itself a clear Mendelian unit (Castle, :15*b*, p. 722).

. . . there can be no doubt that only a single genetic factor is here involved (Castle, :16, p. 95).

It is precisely this last named category of cases [a single factorial basis undergoing quantitative variation] which alone can explain our rat results (Castle, :15*b*, p. 725).

Energetic attacks have been made on the interpretation Castle has given to his results, and certain unwarranted criticisms have been duly answered. That the theory of multiple factors may be applied to the results as published in 1914 in the Carnegie Institution Publication No. 195 was indicated therein by Castle, and further emphasized by Muller (:14). Most of the criticisms of the experiments with the hooded rats have been based on the

generalizations that have been made, and not directly on the data. In this paper the writer has used the original data, making verifications where possible, and recalculations of many of the constants. A few inconsistencies and arithmetical errors were found.

That there may be no misunderstanding as to the nature of the multiple factor interpretation, the following scheme is suggested. In the absence of any factor that determines uniform color—in other words, in the presence of two doses of the factor for hoodedness—the amount of pigment on hooded rats may be influenced by several factors. Some of these increase, others reduce, the pigmented area. The factors that increase the pigmented areas (plus factors) form Mendelian pairs (allelomorphs) with the factors that decrease the pigmented areas (minus factors). Dominance is lacking; if a factor is contributed to the zygote by both parents, that factor has more power than if it had come from only one parent. Furthermore, environment, or other conditions which are not inherited, being outside the germ plasm, have such a modifying influence on the pigmented areas that the potential differences between individuals determined by different combinations of factors in the germ plasm, are frequently concealed. It is not pretended that this is the only application of the multiple factor hypothesis that can be made, but it is hoped that the following arguments may become more significant with this suggested application in mind.

The writer herein undertakes to show that the conception of multiple factors may still be applied to Castle's data. The points that favor the multiple factor interpretation of the rat experiments, as well as certain objections that are said to definitely disprove this theory, are brought together in the following paragraphs.

Points Favoring the Multiple Factor Interpretation

1. The gradual divergence of the plus and the minus races may be brought about by the sorting out of groups

of different factors. It has been generally recognized that this is a possible conception. The following authors have considered this point: Castle and Phillips, :14, Muller, :14, Hagedoorn, :14, MacDowell, :15.

2. The hybrid ancestry of the original parents affords a source for a large amount of heterozygosis. It is the reduction of this heterozygosis that selection is supposed to accomplish, in separating the two races, plus and minus.

3. Such a reduction of heterozygosity would be hindered by the large number of matings made between rats less closely related than brother and sister. This point has been discussed by Muller.

4. By breaking the correlation between the soma and the germ plasm, environment has probably played a large part in hindering the reduction of heterozygosity. Apparently it has been assumed that there is a close relationship between the germ plasm and the soma, that the smooth curve of the averages in successive generations proves that the germinal variations, to which the rise in the curve is due, are small and constantly occurring. But, since the rôle of environment is not known, the gradual advance in the averages can not prove anything as to the size of the germinal variations. The presence of regression makes it clear that environmental, or extra-germinal, influences are active in producing variability in the hooded pattern. Regression is really a measure of the degree of independence of the soma and germ plasm. Regression expresses the inverse relationship between the actually tested breeding possibilities and the appearance of the parents. There can be no question as to the activity of environmental influences; of their power and nature nothing seems to be known. The immediate environment of the undifferentiated blastomeres is probably as important a factor in the final appearance of a character as the germ plasm itself. The factors in the germ plasm are like chemicals that will react in a definite way in connection with certain other chemicals; when

different ones are combined with the first ones, the results may be reversed. Now to study one variable (germ plasm) through a measure (soma) influenced by a second variable (environment) will seldom give correct results if the effect of the second variable is not clearly recognized and discount made for its influence. In the present case, it appears that the curve of the averages can only show the degree to which the variations due to environment and the germinal variations tend to go in the same direction. That there is a rise in the curve shows that, on the average, they are a little more likely to agree in direction than to contradict each other. On the other hand, since the environmental variations can not be accounted for and eliminated, the curve gives no information as to the actual or relative potencies of either set of variables. That there are no fluctuations in the curves may have been assumed to prove that environment is constant and therefore does not demand consideration. But this conclusion can not be safely drawn from the facts. The curves probably do mean that, when generation is compared with generation, the variations of the environment are cancelled out; they mean that these environmental, or extra-germinal, variations occur within a generation, and probably within a family or within the gonads of the parents. Environment might well be ignored were the ultimate question to be answered, "How much can selection change the average grade of hooded rats?" But this is not the main question. The question to be answered is, "What is the nature of the changes in the germ plasm?"

In view of all this, one can find slight justification for assuming that the germinal variations were small and constantly occurring. It seems entirely possible that the environmental, or extra-germinal influences were strong, perhaps even more effective than the germinal constitution. In this case, there would be no need to assume a very large number of factors to find a multiple factor explanation for the slow advance wrought by selection.

Such strong environmental influences would, for the most part, effectively confuse the various combinations of germinal factors, and selection would continue to produce slight advances for a long time.

5. Castle has explained (Castle and Phillips, :14, p. 24) the significance of the "... observed reduction of variability" for the multiple factor interpretation; he stated at the same time that "... extensive modification through selection is possible without any marked falling off in variability." Since the observed reduction

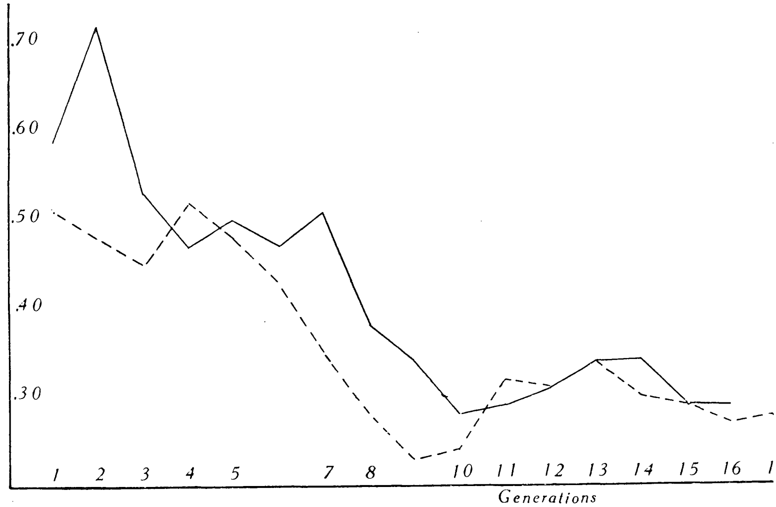

FIG. 1. Standard deviations of the plus race (solid line) and minus race (broken line) in the various generations of selecting. Ordinates represent standard deviations in terms of grades of hooding; abscissæ represent generations of selecting. Calculations made from the data as given by Castle and Phillips, 14, in Tables 1–13 and 16–28.

in variability is not considered to be marked, Fig. 1 is presented to show the facts graphically. The standard deviations plotted in this graph have all been calculated directly from the data, and in several cases they differ slightly from those given by Castle. The decrease in variability that is shown by this figure is the expected result of reduced heterozygosity accompanying continued selection.

6. The question of the rate of advance has been an-

swered by the statement that "no slowing up is observable in the rate of change of the racial character under selection either plus or minus" (Castle, :16, p. 96). This is assuredly a very vital point in the contention that multiple factors will not explain the results. For, if the rate of advance has not fallen off, and if, during seventeen generations, each selection has been as effective as the preceding one, it certainly would look as though this progress were due to constantly varying germ plasm, and not to the sorting out of certain groups of factors. Were a sorting out of factors going on, each advance would restrict the possibilities for further advances, so that in a series of selections the rate of advance would decline.

In Castle's "Heredity," page 122, Fig. 41 are shown the curves of the averages of the first eight generations of the plus and minus races. These curves begin with the average of the offspring that appeared after the first selection. From this point on, the advance shown by the curves is gradual. But should not the advance resulting from the first selection be recorded? The average of the first selected generation was not the point of departure. To show the advance resulting from the first selection, the first point of the curve must give the average of the hooded race before the first selection. Unquestionably the difference between the average of the unselected race and the first selected generation was an advance due to selection, yet this advance is apparently ignored in the statement quoted above, as well as in the figure cited. The first selection resulted in a very much greater advance than any other single selection in the whole series. It took the ten subsequent selections to separate the means of the two races as far as the first selection separated them. If each selection had produced a like advance, the eleventh generation of selection should find the averages of the two races eleven times as far apart as they were after the first selection instead of twice as far apart. Failure to consider the advance due to the first selection has concealed one of the most striking features of the

whole series of experiments, namely, that the first selection brought about an immediate and abrupt establishment of two races with means 3.05 grades apart. The greatest divergence between the two races due to a single selection in all the following generations was 0.64 grade. This followed the third selection. In the second generation there was a reduction of the average of the plus race. Castle explains this as follows:

> To obtain larger numbers of offspring, several new pairs were added to the experiment in this generation which did not appear in Table I either as offspring or parents, but which were derived from the same general stock as the parents of generation one (Castle and Phillips, :14, p. 9).

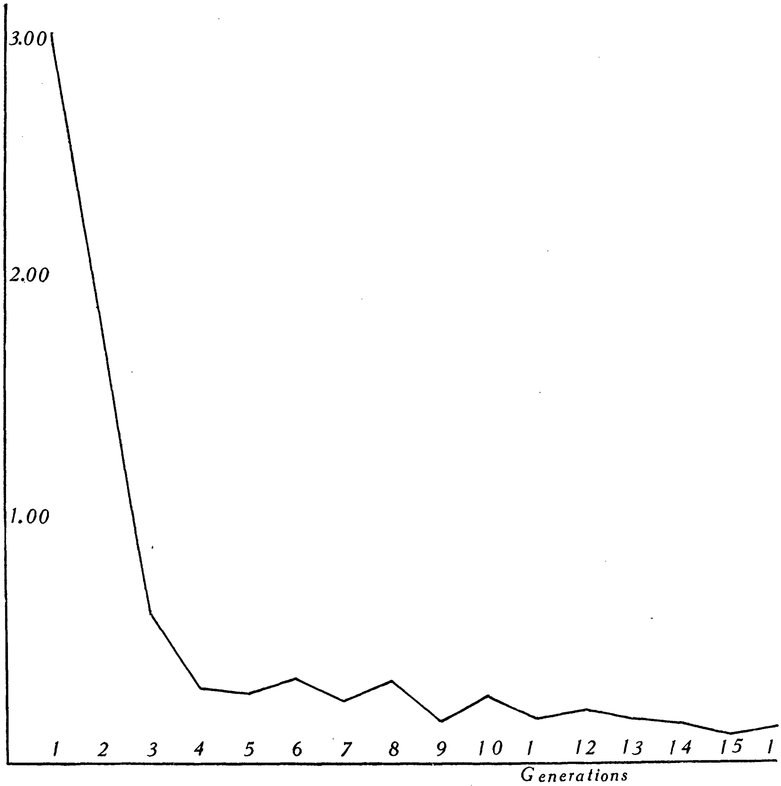

FIG. 2. Rate of divergence of the means of the two selected races in the various selected generations. The point for the second generation has been omitted from the curve; see explanation in text. Ordinates represent grades of hooding; abscissæ represent generations of selecting.

After the third generation there is, in general, a gradual decline in the effectiveness of selection, till in the fifteenth generation the advance is 0.12. In support of this statement, which stands in direct disagreement with the quotation at the head of this section, Fig. 2 is offered. In this figure the ordinates represent the increases in the differences between the two racial means in successive generations. Since the decline in the second generation, as explained, seems to have no immediate significance, this point has been omitted from the curve. The advance in the third generation has been calculated from the difference between the two races in the first generation, which of course gives a slightly smaller advance than if the difference in the second generation had been used. The greater part of the falling off occurs in the plus race, but both races show the same general tendencies, namely, a sudden advance as the result of the first selection, with much reduced advances following subsequent selections. It may be supposed that there was a greater degree of heterozygosity in the parents selected to start the minus race than in those selected to start the plus race. This might explain the smaller initial advance in the minus race (one unit as compared with two in the plus race) as well as the more prolonged and slower subsequent advances.

7. Regression, as Castle uses the term, is the difference between the averages of the selected parents and their offspring. It is due to the imperfect correlation of two variables, intra-germinal and extra-germinal differences, and so, as stated, it forms a gauge of this relationship. Its amount will be reduced by a reduction in the amount of variability of either variable. In the later generations the regression is reduced. We see no reason to suppose that environment as a whole acts any differently in different generations. Therefore this reduction in the amount of regression becomes further evidence in support of the supposition that the germ plasm is more uniform, more homozygous in the later generations.

8. As Muller has correctly reasoned, successful return selections would be expected on the multiple factor view, supposing the races were still "heterozygous even after generations of selection" (Muller, :14, p. 571). It may be added (MacDowell, :15, p. 95) that the failure of a return selection to reduce the average, as long as the advance selection was progressing, would be strong evidence against the multiple factor interpretation. As long as there remained any heterozygosity in the race, both advance and return selection should succeed in moving the averages.

9. In no case should return selection lower the averages at a rate faster than advance selection was raising them at the same time. That is, a certain degree of heterozygosity will permit a certain rate of advance or decline of the averages. The return selections that were started after several advance selections did not show a decline that could be compared with the sudden advance that occurred in the first selected generation. The plus selections had reduced the heterozygosity and had thus set closer limits on the effectiveness of return selection, as well as of further advance selection. So return selections from later generations should be less effective than return selections from the earlier generations. In the plus race, generation 7, there is a difference of .84 between the average of the offspring of rats selected to continue the plus race and the average of the offspring of rats chosen for a return selection. In generation 12 the corresponding difference is .60. In the minus race the difference between the averages of offspring from high and low grade parents in 7, 8 and 9 average .50. In generation 12 the difference is .28. The numbers of rats are very small in most cases, but it is interesting to note that as far as they go they seem to show that return selection is less effective in the later generations than in the earlier ones.

10. Castle has shown that the increase in variability in the first generation of a cross between the plus and minus races may be considered an indication of segrega-

tion, therefore, of heterozygosity in the races (Castle and Phillips, :14, p. 30). Muller (:14, p. 573) repeats Castle's suggestion that the further increase in the variability in the second generation of this cross argues for the supposition that the two races differ in regard to several factors. This is a logical interpretation uncontested by the facts, though of course it is not proved. On the other hand, it has been proved, by the crosses between the two races, that they are not distinguished by just one Mendelian unit. Now if the increased variability in the F_1 of the cross between the plus and the minus races be due to heterozygosis in these races, and if selection is reducing this heterozygosis, crosses made after the races had been selected for a longer time should give less variability in F_1. The figures show this to be the case. If there is less variability in F_1, in later generations there should correspondingly be less variability in the F_2. We find:

F_1 from cross plus by minus, generations 5 and 6 = S. D. 0.71; generation 10 = 0.60

F_2 from cross plus by minus, generations 5 and 6 = S. D. 1.01; generation 10 = 0.87

11. The reductions in the averages of both the plus and minus races after crossing with wild or Irish, first led Castle to consider a factorial interpretation (Castle and Phillips, :14, p. 25). Muller (:14, p. 574) has fully restated the bearing of this on the multiple factor theory. The cross has apparently undone selection to some extent by restoring some of the factors that had been selected out in forming the two races; the cross has increased the heterozygosity of the extracted hooded rats, returning plus factors to the minus race and minus factors to the plus race.

In the light of the above interpretation, the conversion of the minus race into the plus race by means of a cross is significant. Selection for increase in pigmentation was started from extracted hooded rats from a cross of minus with wild. The first generation of this selection made as sudden an advance as the first generation of selected plus rats did at the beginning of the experiment.

It is to be observed that a cross makes a profound difference in the effectiveness of return selection. Crossing has so modified the germ plasm that rats from the minus race immediately, without any gradual return to the "0" grade, repeated the history of the plus race. Further, plus selection was carried on in this new race. Castle (Castle and Phillips, :14, p. 21) emphasizes the fact that this race is free from the objection urged against the main experiment, namely that the closest inbreeding was not carried out. Further interest in this closely inbred race lies in the fact that, although it starts out with a curve almost identical with the first generations of the plus race, the rate of advance falls off faster than it does in the main plus race. One may suppose that the cross produced an F_2 in which some rats had a degree of heterozygosity similar to that which existed in the original unselected stock; a closer inbreeding reduced the heterozygosity more rapidly.

12. The earlier generations of the plus race when crossed with wild are only slightly reduced in pigmentation. In Table 43, Castle and Phillips (:14, p. 48) show, among other things, the averages of hooded grandchildren extracted from a cross with Irish. In comparison with these are placed averages specified to be of offspring from the same grade parents and the same generation of the uncrossed selected race. References to the proper tables of the uncrossed selected races show practical agreement with the averages as quoted in this Table. In Table 42, which gives corresponding results of crosses with wild, three of the averages of the uncrossed races are taken from the same generation as the parents crossed, and three seem to be taken from the following generation. It is a matter of importance to have correct standards for judging the modifications due to crossing. There might be a question whether one should use the average of the generation from which the parents came, or the following one; but in either case the use should be constant. Although the averages of

the generations from which the parents came have been used for comparison in eight of eleven crosses, it appears to be a more fair procedure to compare the averages of the offspring produced by parents of the same grades and generations as used in the crosses. Suppose the hooded parent crossed was grade 2, from the fourth generation, then the average of the *offspring* from parents of grade 2 from generation 4 should be compared with the hooded offspring in F_2. In other words, the average to be used for comparison would be found in generation 5. On this basis the comparisons shown in Table I have been made. Returning now to the statement at the head of this section, that when crossed with wild, the earlier generations of the plus race are only slightly reduced in pigmentation, this table shows that, when third generation parents were crossed, the extracted hoodeds were lowered .04; when fifth and sixth generation parents were crossed, the lowering of their hooded grandchildren was greater, .17; when the hooded parent came from the tenth generation, the average of the hooded grandchildren was lowered .76.

TABLE I

CALCULATION MADE FROM DATA FROM CASTLE AND PHILLIPS, :14, TO SHOW THE EFFECTS OF CROSSING ON THE AVERAGES AND STANDARD DEVIATIONS OF THE EXTRACTED HOODEDS

Numbers in brackets are those given by Castle and Phillips.

A. Comparisons of the Averages of Extracted Hooded Rats with the Averages of the Offspring of Hooded Rats of the Same Grade and Generation as the Hooded Rats Used as Parents in the Various Crosses.

	Wild by Minus Race		
Generation from which hooded parent came	2½	6	10
Average grade of F_2 hoodeds	+ .31	+ .25	+ .25
Average grade of uncrossed hoodeds	— 1.18	— 1.72	— 2.12
Average grade of uncrossed hoodeds as published	(— 1.20)	(— 1.59)	(— 2.05)
Raised by cross	1.49	1.97	2.37

	Wild by Plus Race		
Generation from which hooded parent came	3	5 + 6	10
Average grade of F_2 hoodeds	+ 2.56	+ 2.97	+ 3.15
Average grade of uncrossed hoodeds	+ 2.60	+ 3.14	+ 3.91
Average grade of uncrossed hoodeds as published	(+ 2.60)	(+ 3.14)	(+ 3.84)
Lowered by cross	.04	.17	.76

	Irish by Minus Race		
Generation from which hooded parent came	3½	4	7½
Average grade of F_2 hoodeds	—.62	—.73	—.94
Average grade of uncrossed hoodeds	—1.28	—1.64	—1.83
Average grade of uncrossed hoodeds as published	(—1.31)	(—1.18)	(—1.75)
Raised by cross	.66	.91	.89

	Irish by Plus Race	
Generation from which hooded parent came	2	3
Average grade of F_2 hoodeds	+1.27	+.95
Average grade of uncrossed hoodeds	+2.10	+2.60
Average grade of uncrossed hoodeds as published	(+1.80)	(+2.50)
Lowered by cross	.83	1.65

B. Comparisons of the Standard Deviations of Extracted Hooded Rats with the Standard Deviations of the Offspring of Hooded Rats of the Same Grade and Generation as the Hooded Rats used as Parents in the Various Crosses.

	Wild by Minus Race		
Generation from which hooded parent came	2½	6	10
S. D. of F_2 hoodeds	1.03	.90	1.18
S. D. of uncrossed hoodeds	.56	.33	.31
S. D. of uncrossed hoodeds as published	(.49)	(.44)	(.24)
Increased by cross	.47	.57	.87

	Wild by Plus Race		
Generation from which hooded parent came	3	5 + 6	10
S. D. of F_2 hoodeds	.50	.52	.45
S. D. of uncrossed hoodeds	.47	.47	.29
S. D. of uncrossed hoodeds as published	(.53)	(.49)	(.36)
Increased by cross	.03	.05	.16

	Irish by Minus Race		
Generation from which hooded parent came	3½	4	7½
S. D. of F_2 hoodeds	.64	.60	.84
S. D. of uncrossed hoodeds	.53	.34	.26
S. D. of uncrossed hoodeds as published	(.48)	(.46)	(.35)
Increased by cross	.11	.26	.58

	Irish by Plus Race	
Generation from which hooded parent came	2	3
S. D. of F_2 hoodeds	.90	.87
S. D. of uncrossed hoodeds	.38	.47
S. D. of uncrossed hoodeds as published	(.75)	(.53)
Increased by cross	.52	.40

If the difference between these selected generations lies in the changed position of the mode of continuous ger-

minal fluctuations, one would have difficulty in accounting for the above facts. If these various selected generations differ in the number of multiple factors they bear, one can easily understand that the reason that practically no modification is apparent when the third generation is crossed, is that the number of plus factors in this generation and in the wild are not very different; in the fifth and sixth generations there may be a few more plus factors than in the wild, and in the tenth generation there are several more.

13. The early generations of the plus race, although only very slightly lowered by crosses with wild, are strikingly lowered by crosses with Irish. In a cross in which the hooded parent came from the second generation, the lowering was .83; when the hooded parent crossed came from the third generation, the lowering was 1.65. Now how may this fact be interpreted? If the change in the means following a cross be assumed to be due to the action of different numbers of factors in the races crossed, it is clear that this particular wild is more like the plus race in regard to its factors than is the particular Irish race. In other words the wild race seems to have more plus factors than the Irish race. When early generations of the plus race are crossed with wild there is hardly any change in the averages of the F_2 hoodeds, because there are about the same plus factors in the wild as in these early generations of the plus race. When these same generations of the plus race are crossed with Irish there is a considerable decrease in the averages because there are fewer plus factors in the Irish than in the early generations of the plus race. Now if the germ plasms of the wild and Irish differ in regard to the number of accessory factors, and if the germ plasms of the plus and minus races differ in this same regard, comparisons of all the crosses between these races should show the following results: crosses between wild and minus should give greater modifications in F_2 than crosses between wild and plus; crosses between Irish and minus should modify the

F_2 hoodeds less than crosses between Irish and plus. More directly, the plus race should be more modified by the Irish, the minus race more modified by the wild. Observation of Table I will show that these results are realized.

As already noted in the case of crosses between the plus and wild races, this table shows that in other crosses the different generations of the selected races are differently modified. After long selection there is more modification as the result of crossing. This generalization is supported by all the averages and all the standard deviations in crosses involving the wild race; it is supported by all but one average and by all but one standard deviation in crosses involving the Irish race. If selection is sorting out different groups of factors in the plus and minus races, crosses made after many selections bring together groups of factors more diverse than when crosses are made after only a few selections. The greater the diversity in the numbers of plus or minus factors in the animals crossed, the more extensive will be the segregation in the second generation. More extended segregation may be expressed by increased variability and by more pronounced modification of the averages of the F_2 hoodeds.

14. The behavior of the "mutant" in crosses with the plus and minus races gives clear support to the multiple factor hypothesis. Castle (Castle and Phillips, :14, p. 29) has clearly demonstrated this point. The "mutant" is a suddenly appearing, quantitatively increased stage of the hooded character, that is controlled by a Mendelian factor. Crossed with the race from which it sprang, the extracted individuals show no change from the uncrossed race, either as to averages or variability; crossed with the other race, modifications were found, equalling those obtained when the two races were crossed together. The newly discovered factor acts independently of the other factors, is not modified by them, and does not modify them. Being the one difference between

the mutant and the plus race at the time the mutant appeared, this factor affords a critical test for the interpretation of the modifications that result from crosses.

Objection to the Multiple Factor Interpretation

One new point since 1914 has been urged against the application of the multiple factor hypothesis to the results. By the strength of this evidence the authors of the rat publication are "forced to conclude that this unit (hoodedness) itself changes under repeated selection *in the direction of selection*"; (Castle, :15*b*, p. 722). The point follows:

> The changes effected by selection show permanency under crosses with wild rats. They change no more nor less than an unselected hooded race does. A first cross of the selected race seems to show a partial undoing of the changes produced by selection, but a second cross made on a still larger scale, involving over 1,000 second-generation individuals, showed no further change of this sort, but instead a return to about what the selected race would have been had no crossing at all occurred (Castle, :16, p. 96).

If the grade of hooding of the plus race is reduced in crosses with wild by the replacement of factors selected out of the plus race, repeated crossing of the modified rats should produce further reduction. On the basis of the above claim that crosses do not produce such modifications in the hooded pattern all the evidence formerly admitted to favor the multiple factor interpretation has been swept aside. No one would claim that a single strongly supported experiment may not upset large amounts of contrary evidence, but in such cases it is of utmost importance to have the validity of the crucial experiment fully supported. Is the claim that crosses do not change the selected races fully supported? The following are all the data we are given on this point:

Extracted hoodeds from
 hooded × wild ....75 rats, average 2.89; regression on grandparents .56
Extracted hoodeds from extracted
 hooded × wild ....263 rats, average 3.33; advance on grandparents .32

Averaging the 75 hoodeds may first be criticized. These include all the extracted hoodeds that came from crosses between the wild and the plus races. The third, fifth, sixth and tenth generations of the plus race are involved. It has been shown that the early generations of the plus race are not lowered very much by the crosses in comparison with the tenth generation, which was considerably modified. Therefore among these 75 extracted hooded rats are some that were lowered by the crosses, but more that were practically unmodified. Moreover, the 263 twice extracted hooded rats came from ancestors that had been selected for at least ten generations. Only 16 of the 75 once extracted rats had ancestors that had been selected for ten generations; the others, having ancestors selected for a shorter time, would be expected to give lower averages. In testing for further lowering in this second cross it would seem unjustified to use an average including rats not lowered by the first cross or rats that had not been selected for an equal number of generations before the crosses. Modified by the above considerations the comparisons stand as follows:

Extracted hoodeds from
tenth gen. plus × wild .... average 3.15
Extracted hoodeds from
extracted hoodeds × wild .... average 3.33
Uncrossed, same generation and
grade as hooded grandparent .... average 3.84

The conclusion has been quoted that the cross of the extracted hoodeds with wild has not carried on a further reduction, but it has shown a return, "to about what the selected race would have been had no crossing at all occurred." Will the above figures support this conclusion? The cross of the extracted hoodeds with wild does indeed give a higher F_2 average than the cross of the tenth generation, but the difference is only slight (.18). These two averages are based on very different numbers. It is entirely possible that a larger number of rats extracted from the first cross would have had a higher average than that of the rats extracted from the second cross; in such

a case the second cross would be said to show further reduction.

Whether this advance in the second cross returns the hooded grade to about what the uncrossed race would have been is a matter of what average is used to represent the uncrossed race. The original hooded parents were the last parents to be selected in this series of crosses. It seems clear then, as above reasoned in another connection, that the average to be used in comparison with the two groups of F_2 hoodeds is that of the *offspring* of uncrossed parents of the same grade and generation as the original hooded parents used in the crosses. If this average be accepted (3.84), it is plain that even after the second cross there remains a considerable difference between the averages of the uncrossed and the twice extracted hooded rats. There is reason to believe that the changes produced by selection *are* modified by crossing and that it has not been finally disproved that further crossing does not cause further modification. So, as far as can be judged from the data at hand, this crucial test does not seem to offer a final blow to the applicability of the hypothesis of multiple factors.

On the other hand, that modification actually does result from crosses is strikingly proved by the conversion of the minus race into the plus by means of a cross. This experiment has been referred to on page 729. Six successive return selections did not return the average of the minus race to the "0" grade. But after minus race rats were crossed with wild, a single selection of the plus varieties raised the average 2 grades above "0."

Summary

By way of recapitulation, the points referred to are summarized as follows:

A. Seventeen generations of selection need not have entirely eliminated modifiers, because,

1. Matings less close than brother and sister have tended to continue heterozygosity;

2. Environmental influences may possibly act in such a way that only occasionally does a selected individual carry germ plasm more homozygous then the average.

B. The implied claim that the facts do not support the supposition that selection has decreased the number of modifiers, or has reduced the heterozygosity in the two races of rats, has been answered by the following points:

1. Selection reduces the variability.
2. The rate of advance declines as selection is continued.
3. Parental regression is lowered by selection.
4. Return selections argue that heterozygosis is still present; they indicate that there is less heterozygosis after longer selection, since selection reduces the effectiveness of return selections.
5. Crosses between the plus and minus races strongly suggest that heterozygosity is still present by the increase in variability in F_1; they also appear to show that there is less heterozygosity in a later generation, since the increase in F_1 is less in a cross after longer selection.
6. Crosses between the selected races and the wild or the Irish race show that more modification appears in the F_2 hoodeds when crosses are made after longer selecting.

The reader is now in a position to judge whether the writer is justified in concluding that there is still a "possibility that other as yet undiscovered factors might be responsible for the apparent changes observed" (Castle, :15, p. 722) and that the claim that "all the evidence we have thus far obtained indicates that outside modifiers will not account for the changes observed" is too sweeping.

DISCUSSION[1]

A great difficulty has been placed on the discussions of this subject by the different terminology used by those holding different opinions. Calling the visible character the Mendelian unit is a striking example of this difficulty. There is a vital difference between a unit character and a factor, which must be constantly recognized if this discussion is to progress.

It is unfortunate that the word *selection* has come to have the significance of a slogan. For the nature of the actual power of selection itself is not in question. What selection is, can be easily defined and agreed upon. If the nature of the changes in the germ plasm could be determined, there would be little disagreement as to what selection could accomplish. Even those who are not considered to be selectionists believe that natural selection is very important in evolution. So the epithets, selectionist and pure-lineist, fail to indicate the difference between the two groups to which they have been applied. It would be quite impossible to divide biologists into two distinct schools on the basis of a subject upon which there are many different shades of opinion. Any such classification would be inaccurate, even if the most precise definitions of the classes were generally accepted. When there are no accepted definitions, and those most clearly cut are offered by individuals in the opposite group (each one realizing the diverse ideas within his own group and wishing to crystallize an opposing view in order to attack it) such classification of opinion is far from scientific. In the present instance, the classification into selectionists and pure-lineists has tended to magnify the differences between investigators. With a desire to try to overcome

[1] Since the writing of this paper, there have appeared papers by Pearl ("Fecundity in The Domestic Fowl and The Selection Problem," AMER. NAT., 1916, p. 89) and Castle ("Can Selection cause Genetic Change?" AMER. NAT., 1916, p. 248) which have a close relationship to the present discussion. It has been considered wiser to leave this paper as written, than to enter the controversy by including discussions of the two papers mentioned.

the exaggerated differences which seem to exist, the following discussion is offered. It is written with no wish to codify or defend the opposing positions, but rather as an attempt to formulate the issue a little more clearly by presenting two views, which appear to have advocates, of the nature of the changes in the germ plasm.

The view to be called the "first" is as follows: The changes in the germ plasm are in the nature of fluctuations, now larger, now smaller, but continuously appearing; they lead in all directions. This is true of all inheritance, whether or not it be factorial (Mendelian) in basis. If it refers to Mendelian inheritance the potential grade of the factor in question, as found in any zygote, acts as a mode about which the fluctuations in potentiality occurring in the next generation are grounded. In other words, although a zygote may include the strongest potential grade of a factor that has appeared, the inevitable fluctuations in this factor that are found in the different gametes formed by this zygote will include, together with those like and weaker than the parent, some with stronger potentialities than the parent.

The view to be called the "second" is as follows: The changes in the germ plasm are discontinuous; they appear fortuitously. They may strike out in almost any direction, as a projectile may be aimed in "any direction," in contrast to the "all directions" taken by the waves of sound when the projectile explodes.

According to the first view, selection would result in modification in any direction the breeder might desire, irrespective of variational tendencies shown by the animal. To maintain conformity to type would require as constant selecting as would be required to obtain divergence. According to the second view, selection could progress only in certain directions, depending on how the germ plasm happened to change; the variational tendencies of the animals would probably suggest these directions. Conformity to type would be considered to be a fundamental phenomenon due to the conservative tend-

ency of the germ plasm to maintain the *status quo*. On the basis of the first view, the external influence (selection) would have major importance in defining the course of evolution; on the second, the internal influence (the inherent nature of the germ plasm itself) would have major importance. In both cases, the nature of the progeny would depend on the nature of the germ plasm of the parents. In both cases selection would be able to modify the race. But in neither case is the origin of the changes in the germ plasm explained. The fundamental causes of evolution are as much a mystery as ever. Grant a certain hypothesis of germinal changes, and selection becomes a more important factor in evolution than when another hypothesis is granted. But even such an increased importance of selection does not give it the value of a fundamental creative cause of evolution.

There has appeared a theory that would give selection still greater importance by saying that selection has the power to build up unit-factors and induce mutation.

> Unit-characters may arise gradually as the result of repeated selection in a particular direction (Castle, :12*b*, p. 280).
>
> In yellow animals, as in blacks, individuals of varing intensity occur the darkest known as reds, the lightest as creams. A complete series of intermediates can be obtained if so desired. If we select any two widely separated stages in this series fairly stable in their breeding capacity and cross these, they Mendelize, *i.e.*, they behave as if they were a single unit-character difference between them. . . . That difference might equally well be *half* as great as it is, or a *quarter* as great, or a thousandth part as great. A monohybrid ratio would result equally in each case, upon crossing the two quantitatively different stages (Castle, :12*a*, p. 358).

Now this may be true for yellow guinea pigs, but the rats clearly demonstrate that it is not true in all cases. The two quantitatively different stages of the hooded pattern represented by the plus and minus races do not result in a monohybrid ratio when they are crossed.

However there has appeared a "unit-character" difference in one of these races of hooded rats. It appeared suddenly, and it Mendelizes when crossed with other

hooded rats. The occurrence of this "mutant" is claimed to have been induced by selection.

It seems to us quite improbable that this plus mutation could have arisen in the minus selection series. We believe that the repeated selection which was practiced had something to do with inducing this change in the plus direction (Castle and Phillips, :14, p. 31).

No reason for such a supposition is given. On the other hand there is clear reason for supposing that such a mutation would be far more easily detected in the plus series if it occurred there. The same mutation occurring in the minus race would perhaps have the same relation to that race as it had to the plus race when it occurred there; since it would lack the extension factors of the plus race, it would have a very different appearance and would probably have a grade not far from "0." It seems that very few rats of this grade were bred or tested. Had this mutation occurred in the minus race and been isolated, it would have been possible to obtain it as it appeared in the plus race, by proper crossing.

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